

Figure 1

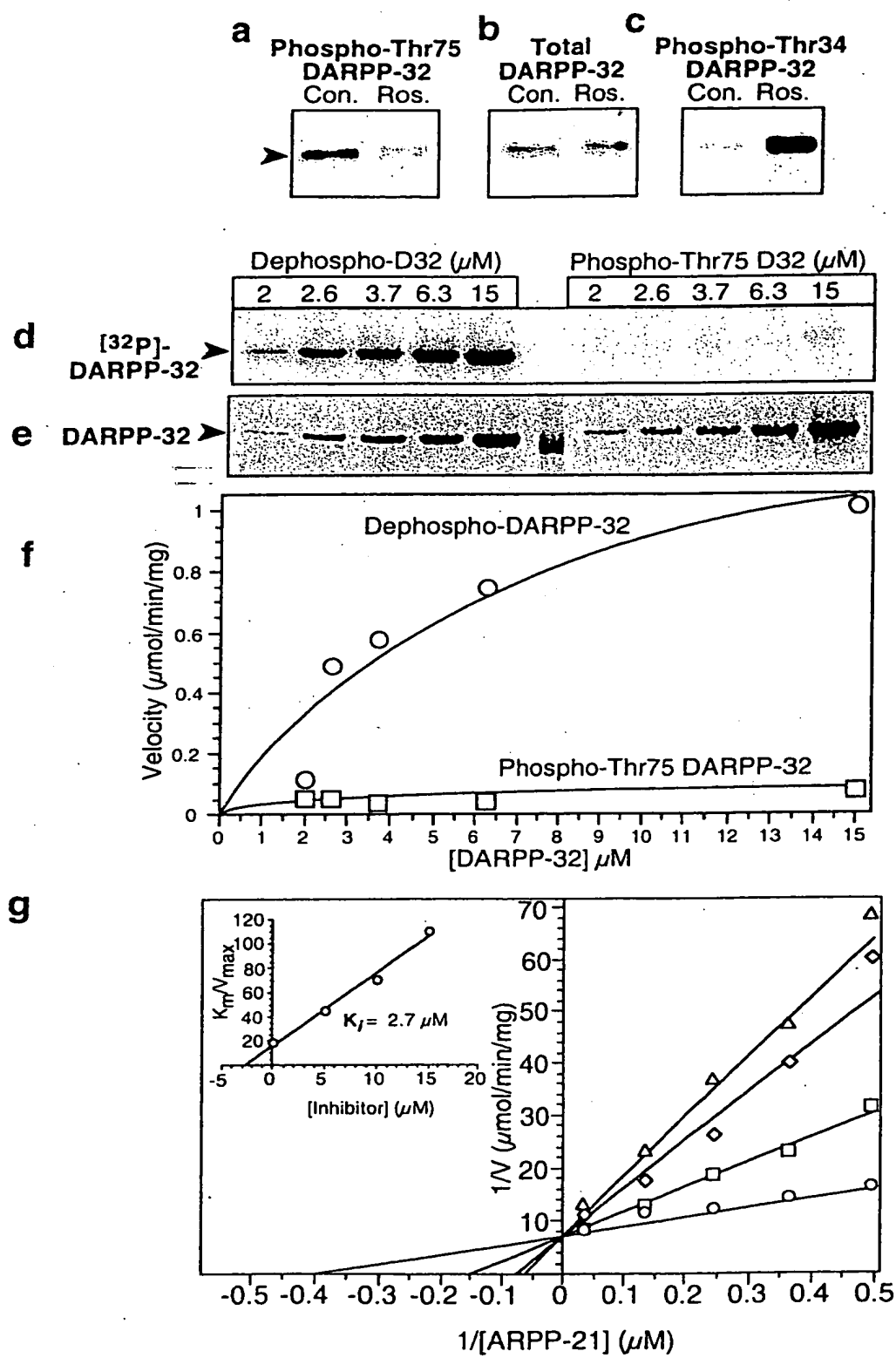


Figure 2

09687959-101300

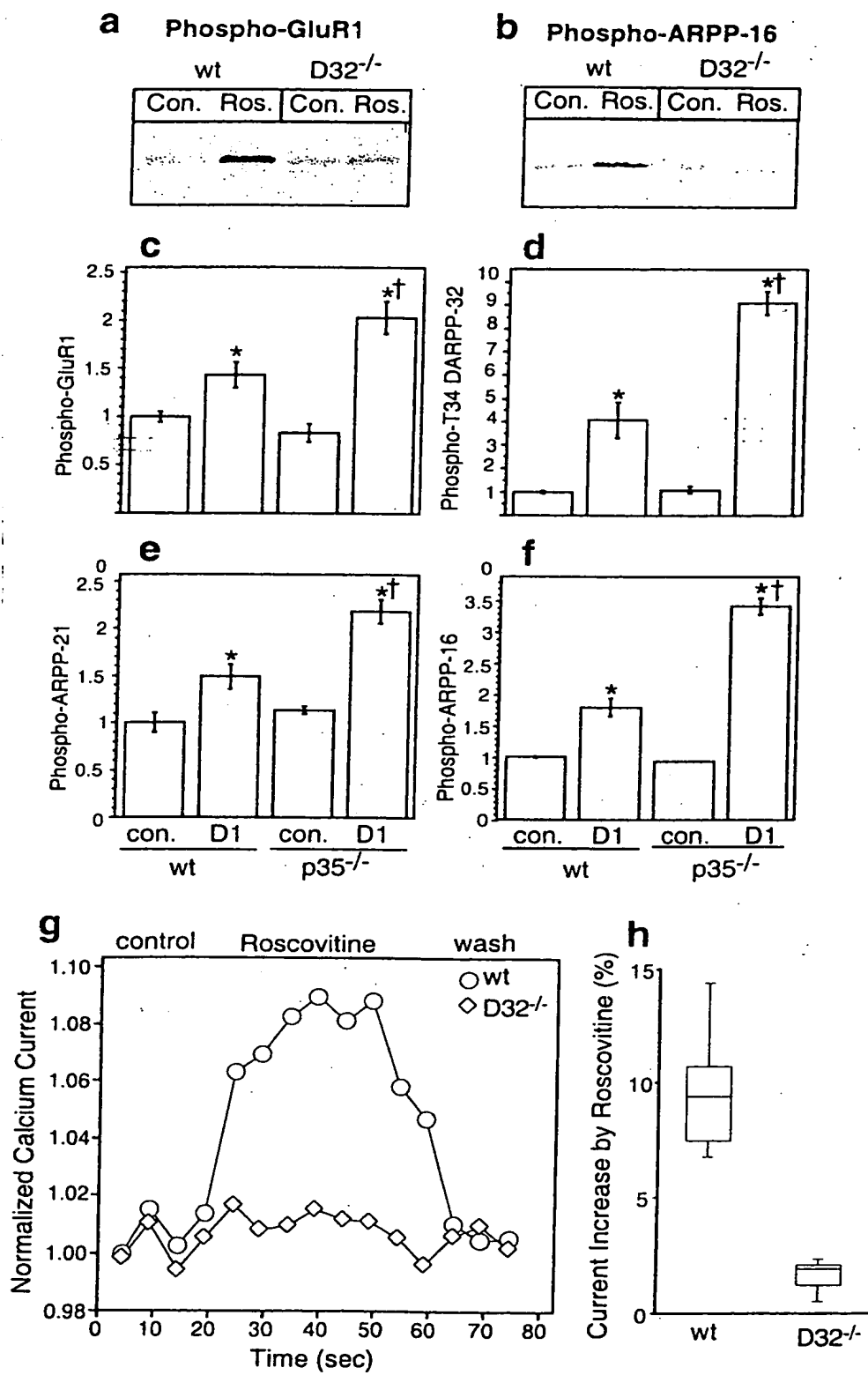


Figure 3

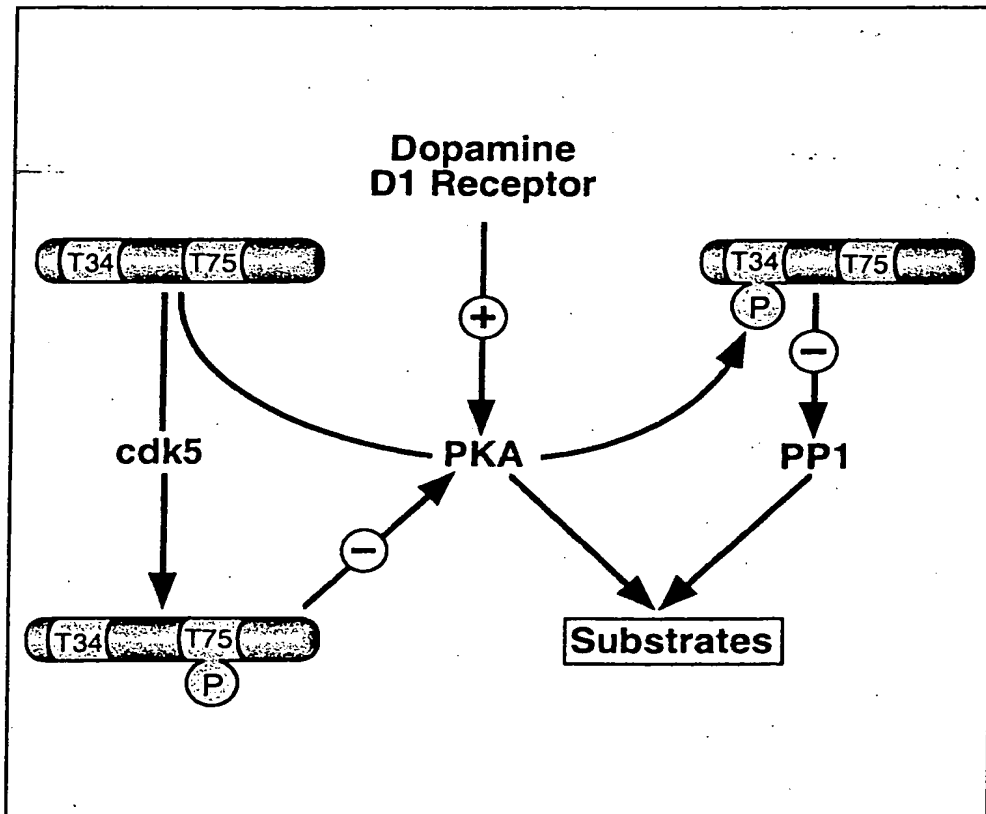


Figure 4A

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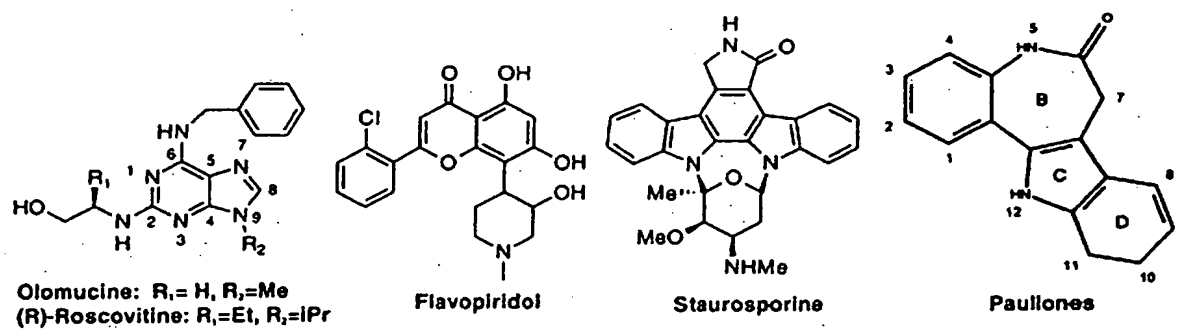
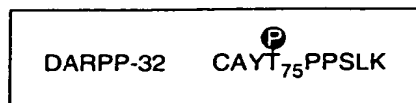


Figure 5

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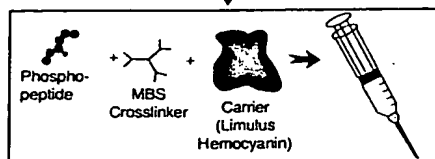
Production of Phosphorylation State-Specific Antibody

A



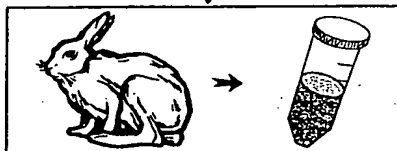
Following determination of the phosphorylated residue, a short synthetic phospho-peptide corresponding to the region surrounding the site is prepared.

B



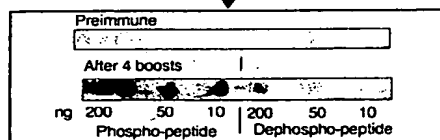
Phosphopeptides are conjugated to carrier protein and prepared for inoculation.

C



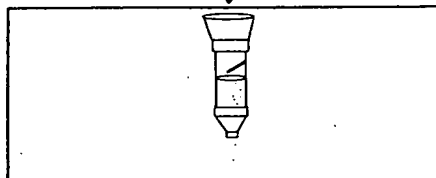
Inoculation, boosting, and bleeding of rabbits.

D



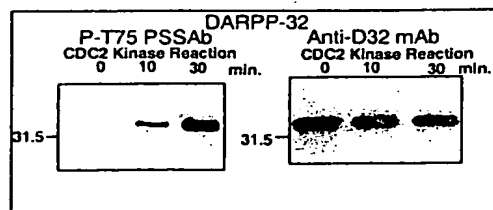
Primary screening of anti-sera by immuno-dot-blotting.

E



Purification of phospho-specific Ab using protein A and phospho/dephosphopeptide affinity chromatography.

F



Confirming specificity by immunoblotting

Steps involved in the generation of phosphorylation state-specific antibodies (PSSAb) are illustrated schematically. Typical results of phospho-peptide dot-blot screening of serum are shown. The bottom panel shows immunoblot results confirming the selectivity of the antibody for phosphorylated protein.

Figure 6

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Human:	1	MDPKDRKKIQFSVPAPPSQLDPRQVEMIRRRRPTPAML	34	PRLSEHSSPEEE	50
Bovine:		MDPKDRKKIQFSVPAPPSQLDPRQVEMIRRRRPTPAMLFRLSEHSSPEEE			
Rat:		MDPKDRKKIQFSVPAPPSQLDPRQVEMIRRRRPTPALLFRVSEHSSPEEE			
Mouse:		MDPKDRKKIQFSVPAPPSQLDPRQVEMIRRRRPTPAMLFVRVSEHSSPEEE			
Human:		ASPHQRASGEGHHLKSKRPNPCAYTPPSLKAVQRIAESHLO	75	SISNLTNENQ	100
Bovine:		ASPHQRASGEGHHLKSKRSNPAYTPPSLKAVQRIAESHLO		SISNLGENQ	
Rat:		SSPHQRTSGEGHHPKSKRPNPCAYTPPSLKAVQRIAESHLO		TISNLSENQ	
Mouse:		ASPHQRTSGEGHHPKSKRPNPCAYTPPSLKAVQ-----HLQ		TISNLSENQ	
Human:	102	ASEEDELGELRELGYPREDEDEDEDEDEDEDEDEDEDE	137	EDSQA	150
Bovine:		ASEEDELGELRELGYPREDEDEDEDEDEDEDEDEDEDE		EDSQA	
Rat:		ASEEDELGELRELGYPREDEDEDEDEDEDEDEDEDEDE		EDSQA	
Mouse:		ASEEDELGELRELGYPREDEDEDEDEDEDEDEDEDEDE		EDSQA	
Human:		TTRGLGLEGPWQRPPPLDESERDGGSEDQVEDPAL-SEP		GEEPQR	200
Bovine:		TTYGQGLEGPWERPPPLDGPQRDGGSEDQVEDPALN-EP		GEEPQR	
Rat:		LTSGQGLEGPWERPPPLDEPQRDGNSEDQGEGRATQSE		GEEP-RH-PT-PP	
Mouse:		PTCGRGLEGPWERPPPLDEPQRDGNSEDQVEGRATLSE		GEEPQ-H-PS-PP	
Human:	P-GR	199			
Bovine:	P-GT	198			
Rat:	ESGT	201			
Mouse:		194			

Figure 7

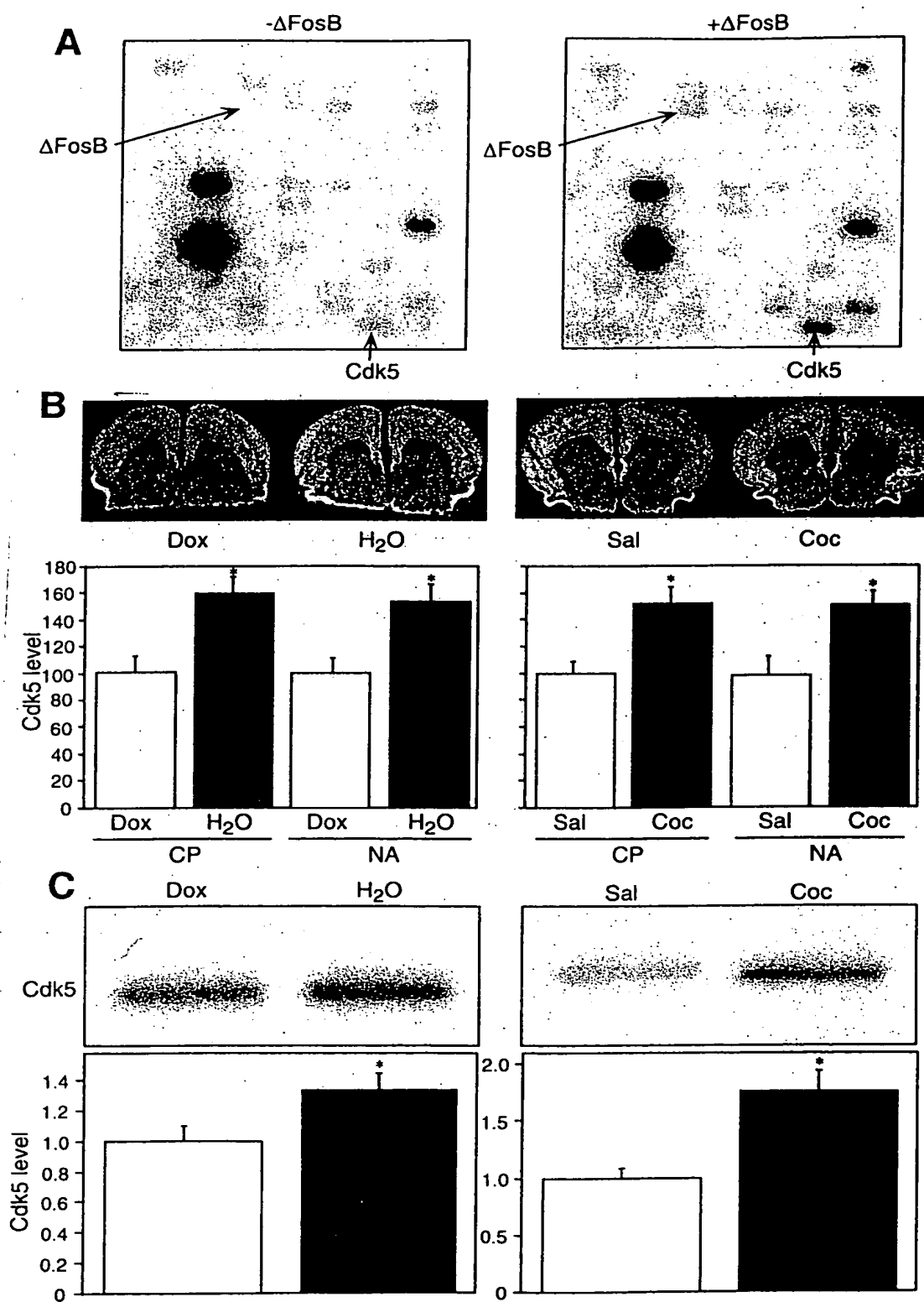


Figure 8

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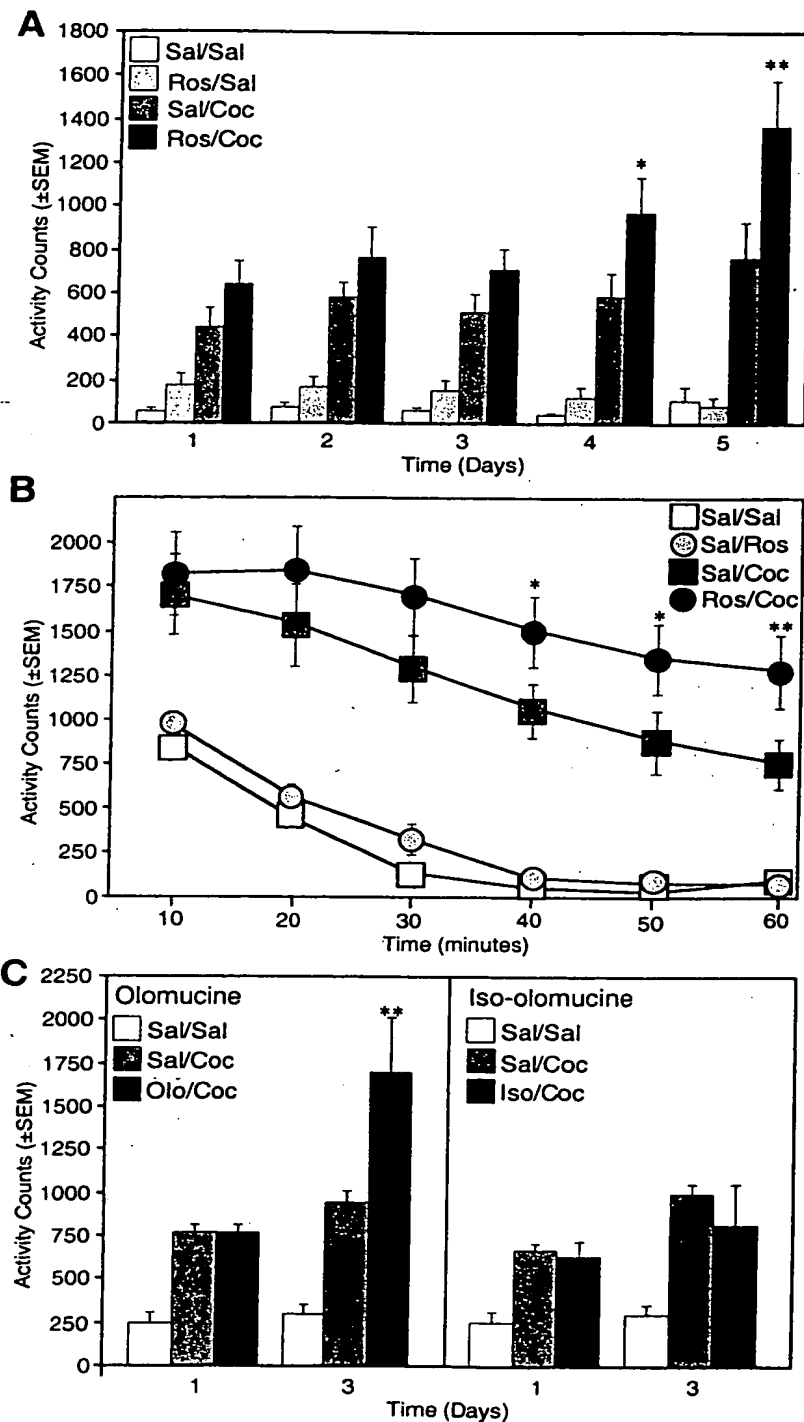


Figure 9

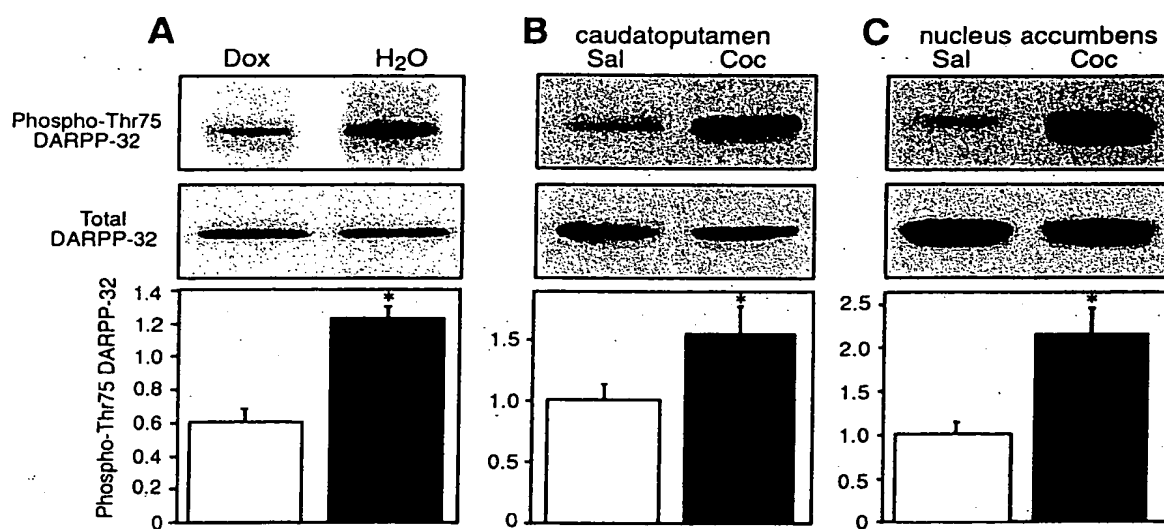


Figure 10

